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SUCCESSFUL CANINE INFECTION WITH CULTURES OF LEISHMANIA INFANTUM
(C. NICOLLE).*

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PRELIMINARY NOTE.

In May, 1903, Major Leishman described peculiar chromatin bodies which he found in the spleen of a patient suffering from the so-called dum-dum fever of Calcutta, and at the time he suggested the possibility of these having a trypanosomal origin. This observation was soon confirmed by Donovan and others, and consequently the so-called Leishman-Donovan bodies have come to be recognized as the cause of Kala-azar or tropical splenomegaly.

In December of the same year J. H. Wright gave an accurate description of similar bodies found in the lesions of Delhi boil or Oriental sore, and like results were obtained about the same time by Marzinowsky. While, morphologically, the Wright bodies are identical with those of Leishman, there is good reason to believe that they represent distinct though related organisms.

A third infection of similar type was recognized by Pianese, in Naples, in 1905. In five out of twelve cases of infantile splenic anemia he found intracellular bodies which resembled those of Leishman. The recent study of this disease of infants by C. Nicolle, director of the Pasteur Institute of Tunis, are of commanding interest in view of the results achieved.

The organisms referred to may be placed, for the present at least, in the genus *Leishmania* created by Ross. They are known, therefore, as:

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Leishmania donovani (Laveran and Mesnil) in Kala azar.

Leishmania tropica (Wright) in Oriental sore.

Leishmania infantum (C. Nicolle) in infantile splenic anemia.

From the beginning of the studies thus briefly referred to, much interest attached to the question of the nature and the systematic position of the Leishman-Donovan bodies. The hypothesis of a trypanosomal origin was strengthened by the discovery of Rogers, in 1904, that the Leishman bodies developed into flagellated organisms when the fluid obtained by splenic puncture was placed in a solution of sodium citrate. The actively motile forms thus obtained were strikingly suggestive of cultural forms of trypanosomes, more especially of the flagellates found in insects, and hence Rogers proposed to designate the organism as an *Herpetomonas*. The observations of Rogers were soon confirmed by others, and the important fact was thus established that the Leishman bodies, usually intracellular, possessed a free, flagellate or trypanosome-like stage. Unfortunately, attempts to grow the organism on blood agar failed and, as it was seemingly impossible to obtain more than one or two subcultures of the flagellated form, the further study of Kala-azar along these lines was blocked for the time being. In view of the results recently obtained by Nicolle with *L. infantum* and *L. tropica*, it is clear that the study of *L. donovani* must be taken up anew.

In the case of infantile splenic anemia Nicolle has shown (February, 1908) that this disease can be reproduced in the dog and monkey by inoculation with the infected tissue and that the human disease is probably of canine origin. Moreover, he was able to cultivate the parasite on blood agar, not only from the human disease but also from the experimental animals and from the naturally infected dogs. Attempts at reproducing the disease by inoculating dogs and monkeys with pure cultures of *L. infantum*, however, failed and he concluded that such cultures, unlike the virus, seemed to be deprived of pathogenic power.

Because of the important questions involved it was very desirable to prove the relation of the flagellated forms to the disease by inducing an infection characterized by the presence of typical Leishman bodies. Ac-

cordingly, having received, through the courtesy of Dr. Mesnil of the Pasteur Institute, March 23, cultures of Nicolle's flagellates (eighth generation); it was decided to repeat Nicolle's experiments on dogs. Since the failure in his tests was probably due to the use of small doses of the culture material, it seemed best to resort to repeated inoculations of large quantities of the flagellates.

In the interval from April 13 to September 21 a dog was given fifteen injections, intraperitoneally, of fresh vigorous cultures of the flagellates. The organism was grown on blood agar at 20 degrees C., and for each inoculation the growth from a large number of tubes (8 to 40) was taken up in citrate solution and then injected. All told 270 cultures were thus utilized. The dog apparently showed no effect other than an occasional leucocytosis. It remained seemingly healthy and repeated microscopic examination of the peripheral blood gave negative results.

On October 9 the dog (weight 11.7 kilos) was killed by bleeding and an autopsy was at once made. Contrary to what might be expected, the spleen was small, tough and leathery and weighed but thirty-three grams. The liver and kidney were likewise unusually hard. Smear preparations made from the spleen, liver, kidneys, lungs, mesenteric glands and red marrow showed enormous numbers of typical Leishman-Donovan bodies. These were at times present in mononuclear leucocytes, from five to one hundred or more being present in a cell, but most of the parasites, however, appeared to be free. There was no evidence of the presence of flagellated forms. A comparison of the Leishman bodies found in this dog with those seen in original preparations kindly sent me by Dr. Nicolle showed them to be identical with those found in the human body. Blood agar tubes inoculated with material from the spleen, liver and bone marrow gave in five days enormously rich cultures of the typical flagellate form.

This experiment, therefore, establishes the susceptibility of the dog to infection with cultures of *L. infantum*. Starting out with the flagellate form, it has been possible to reproduce the characteristic Leishman-Donovan bodies, free as well as intracellular, and, moreover, the recovery of the typical flagellates by cultural means, from the organs of the infected dog, completes the evidence regarding its relation to the disease. It is

interesting to note that the successful infection of dogs by means of pure cultures furthers the prosecution of the study of this disease in laboratories remote from regions where the disease prevails. It is probable that like results will be obtained in the case of Kala-azar and Oriental sore. Further studies on the infectivity of experimental animals are now under way.

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